

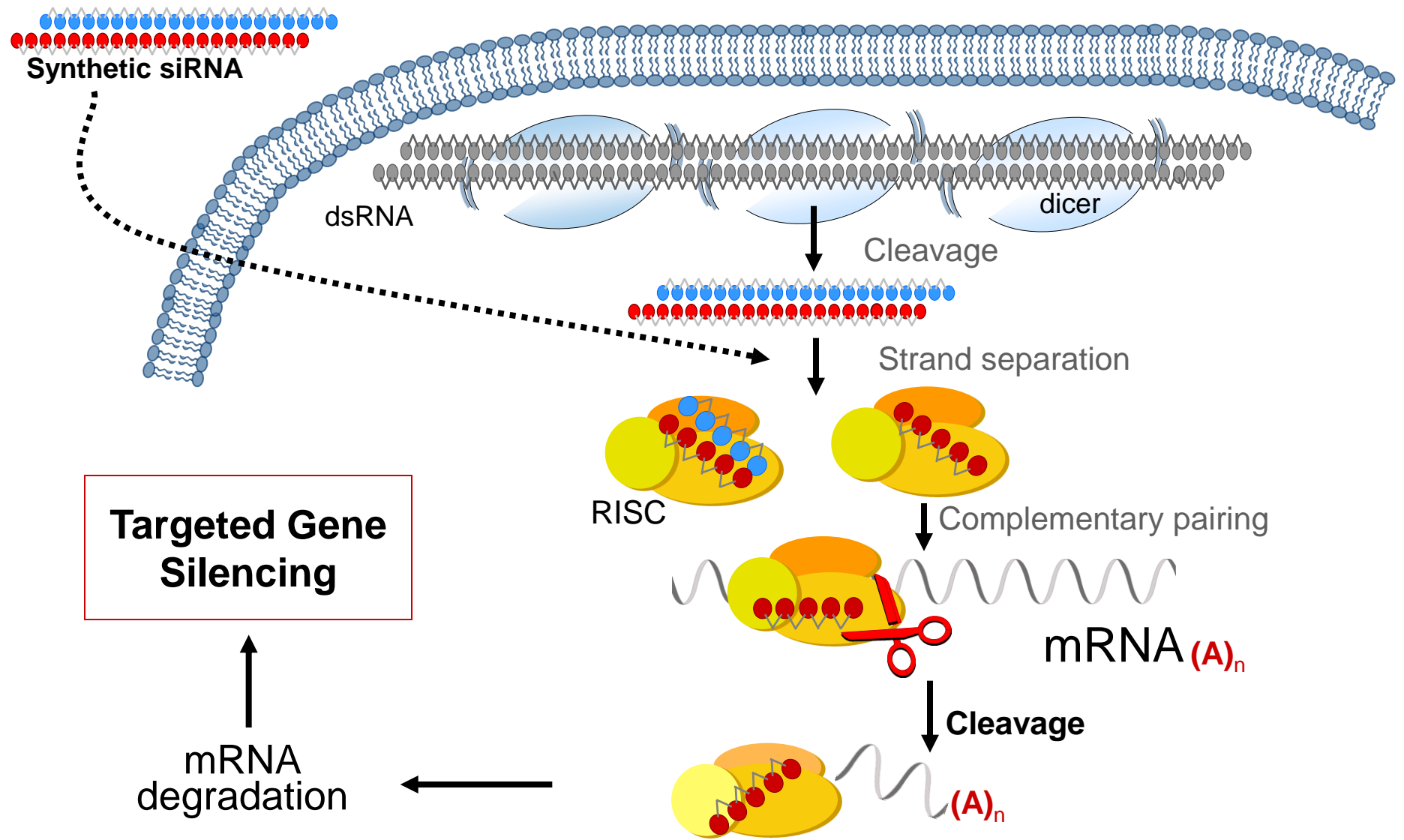
Phase 1 Trial of ALN-VSP in Cancers Involving the Liver

Annual Meeting of the Controlled Release Society
August 2, 2011

- ALN-VSP: Background
- Phase 1 Trial Study Design
- Safety Data
- Tumor Response Results
- Pharmacodynamic Data
- Summary



Mechanism for RNA Interference (RNAi)

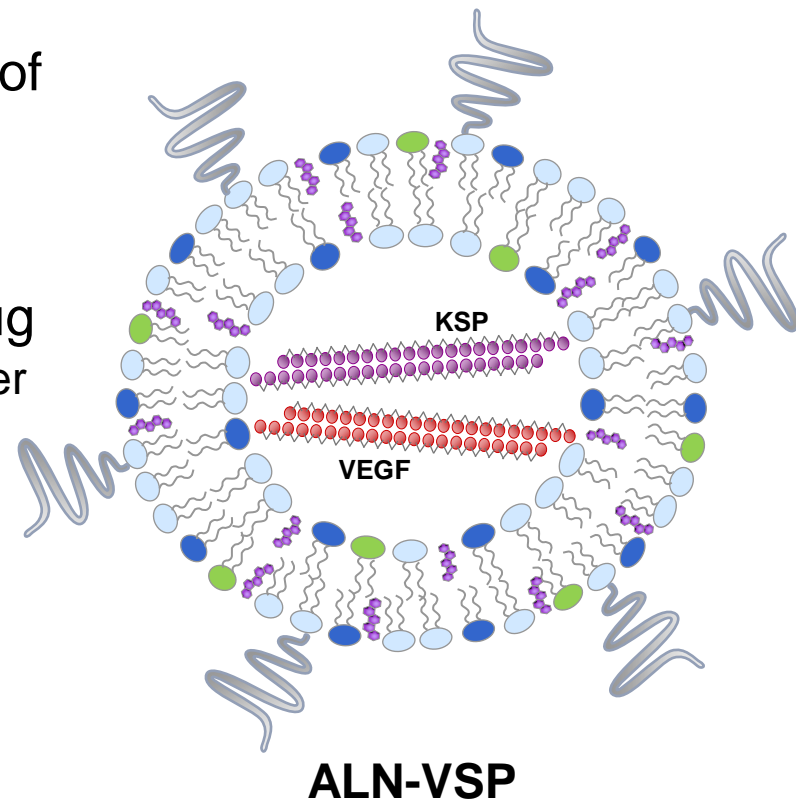


Liver Cancer Program

ALN-VSP

RNAi to treat primary and secondary liver cancers

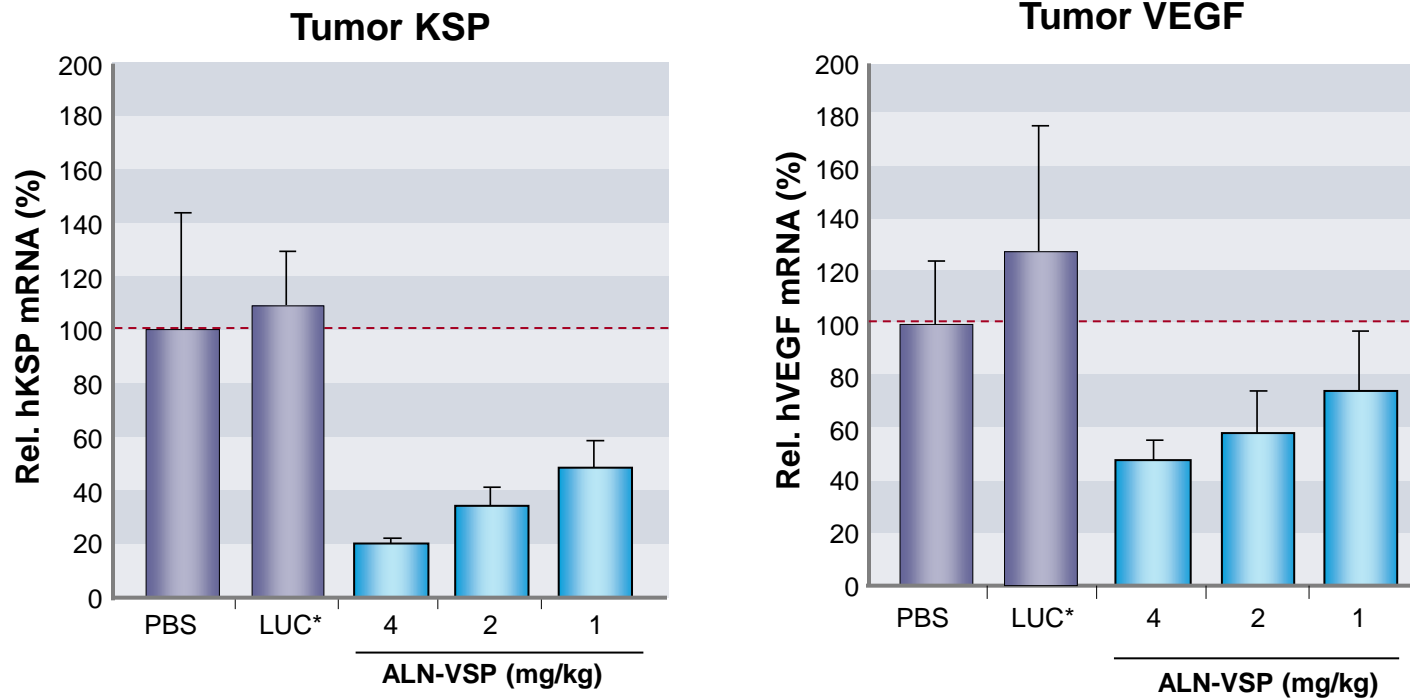
- Prevalent solid tumor and common site of metastatic disease
 - » ~700,000/yr: Incidence of HCC worldwide
 - » ~500,000/yr: Patients with liver metastasis
- ALN-VSP is first dual-targeted RNAi drug
 - » Targets two distinct genes involved in cancer pathways
 - Proliferation: Kinesin Spindle Protein (KSP)
 - Angiogenesis: VEGF
 - » Lipid nanoparticle (LNP) formulation
 - From Tekmira Pharmaceuticals
- Phase I clinical trial for liver cancer
 - » Data presented at ASCO Annual Meeting in June 2011
 - » Study completed July 2011
 - » Multiple patients continuing on extension study



ALN-VSP Efficacy *in Vivo* in HEP3B tumor model

ALN-VSP reduces expression of tumor KSP and VEGF

- Human KSP or VEGF mRNA levels derive from Hep3B tumor

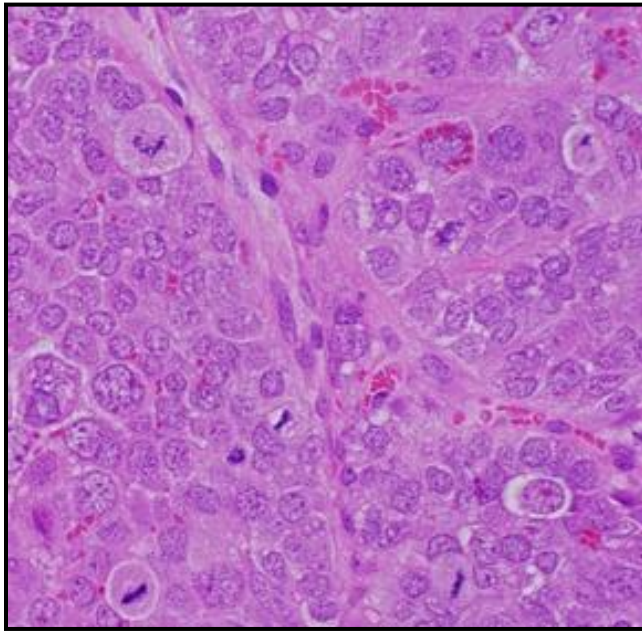


Orthotopic Tumor Models (Hep3B) treated 20 days post seeding;
Single IV injection VSP or 4 mg/kg control siRNA

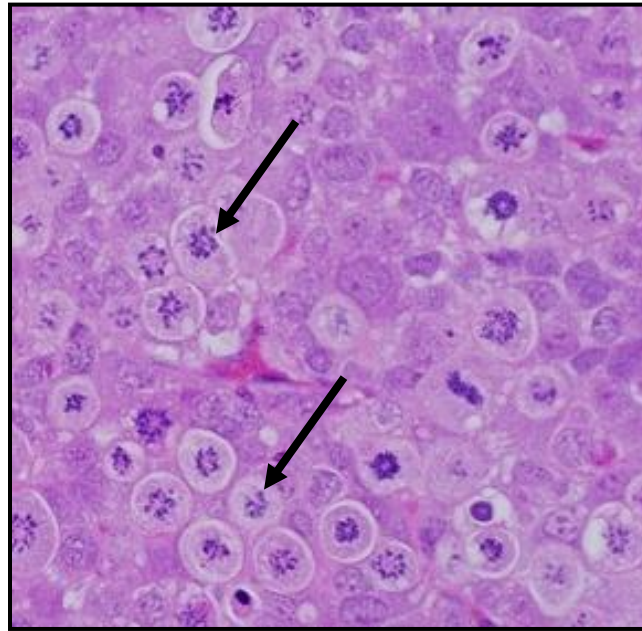
ALN-VSP Efficacy *in Vivo* in HEP3B tumor model

Cell Cycle Arrest *In Vivo* with ALN-VSP

- Single IV bolus injection of ALN-VSP or control siRNA 21 days post seeding
- Mitotic arrest (monoasters) clearly detected in VSP-treated animals



Control siRNA (2 mg/kg)



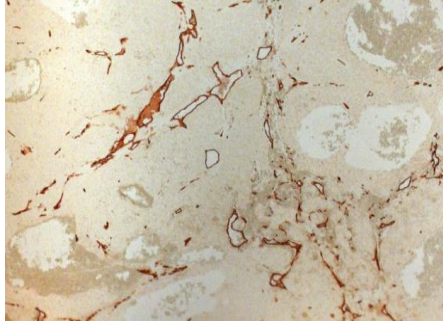
ALN-VSP (2 mg/kg)

Orthotopic tumor model (Hep3B) treated 21 days post seeding;
single IV injection 2 mg/kg VSP or control siRNA

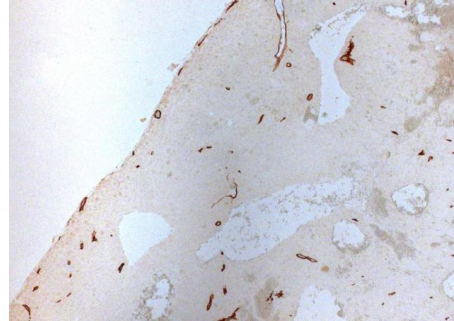
Anti-Angiogenic Effect of Anti-VEGF siRNA

Reduction In Microvessel Density

CD34



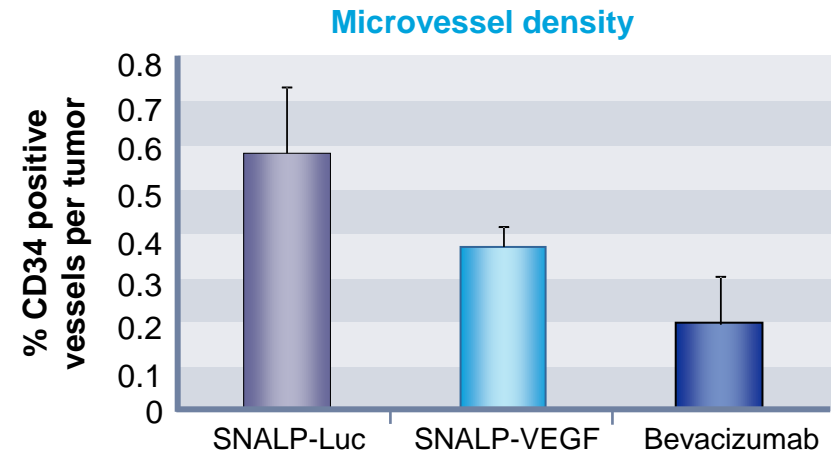
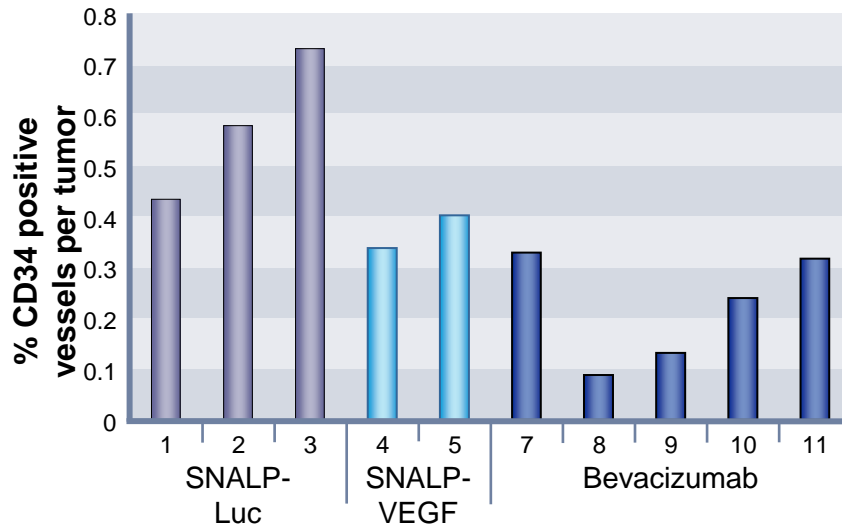
SNALP-Luc



SNALP-VEGF



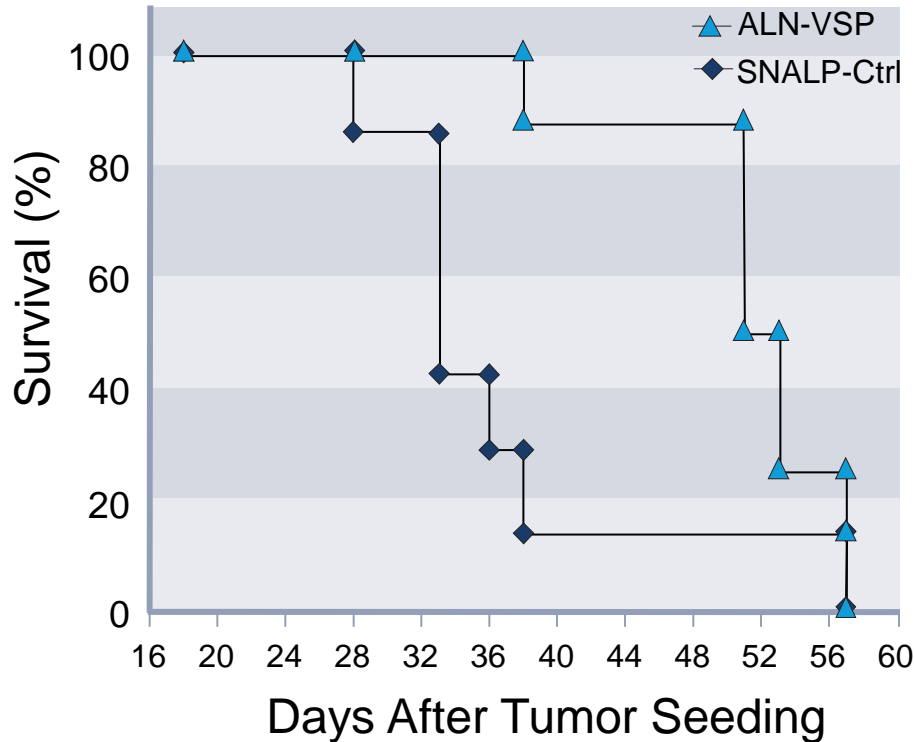
Bevacizumab



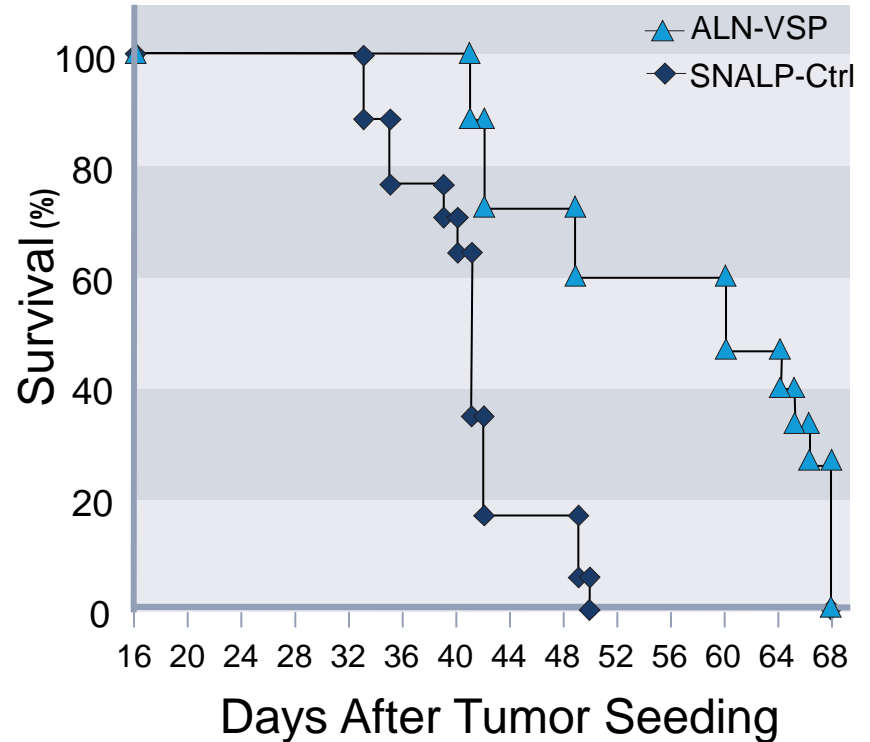
- Orthotopic tumor model (Hep3B) treated 10 days post intrahepatic implantation;
- SNALP-VEGF vs SNALP-Luc at 6 mg/kg & Bevacizumab (Avastin®) at 5 mg/kg IP, 2x per week for 3 weeks

Prolonged Survival With ALN-VSP Treatment

Study 1



Study 2



Orthotopic Tumor Model (Hep3B) – Treated 18 days post seeding;
IV bolus injections of 4 mg/kg VSP or control siRNA 2x/wk for 3 wks

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ALN-VSP Phase I Study Objectives

Primary

- Evaluate the safety and tolerability of ALN-VSP

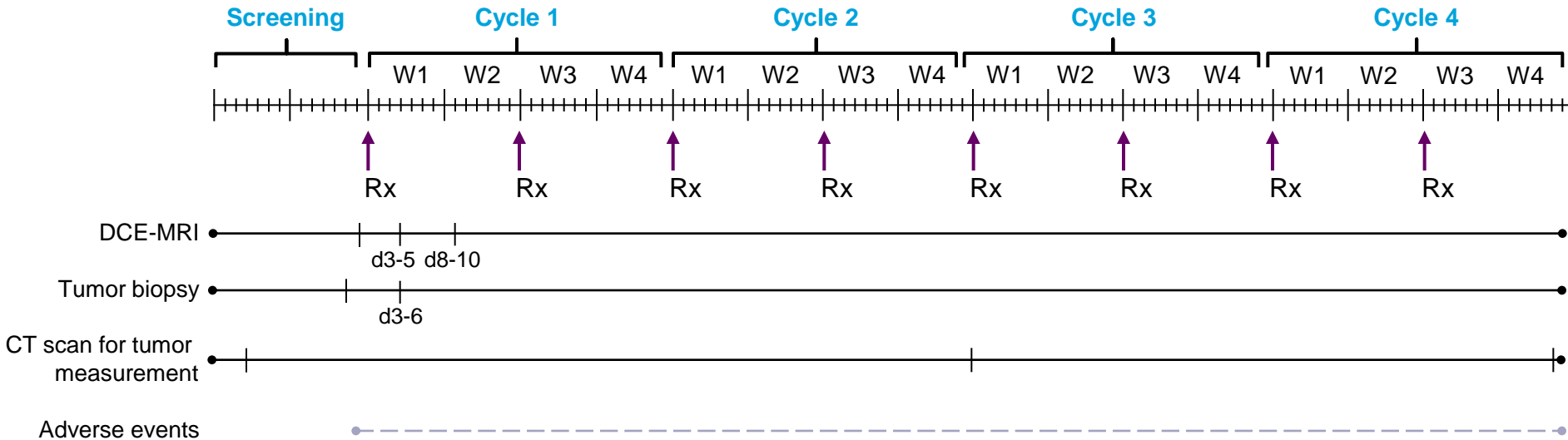
Secondary

- Characterize PK
- Assess for evidence of antitumor/antiangiogenic activity
 - » Tumor response rate, change in tumor blood flow on DCE-MRI

Exploratory

- Analyze voluntary tumor biopsies for drug levels and for evidence of RNA interference using 5' RACE assay

ALN-VSP Phase I Study Design



Dose levels and dosing schedule

- 0.1, 0.2, 0.4, 0.7, 1.0, 1.25, 1.5, 1.7 mg/kg
- 3 + 3 cohort design, expansion phase of 10 pts at MTD
- 15-min IV infusion q2 wks; premed with steroids, H1 and H2 blockers, acetaminophen
- Cycle = 2 doses (1 month), tumor measurements after every 2 cycles, treat until disease progression
 - » ALN-VSP-002 extension study for pts remaining on study beyond 4 cycles (8 doses)

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Demographics and Dosing

- N=41
 - » Dose escalation (0.1-1.5 mg/kg): N=31
 - » Expansion phase at 1.0 and 1.25 mg/kg: N=10 (5 per dose level)
- Trial completed end of July 2011; 5 patients remain on extension study
- Median age 57 (range 34-78)
- Male: Female = 17:24
- ECOG performance status 0/ 1 (%): 44 / 56
- Average # of prior regimens for metastatic disease: 4.3 (range 1-13)
- Prior chemotherapy/anti-VEGF therapy (%): 88 / 61
- Liver/Extrahepatic metastases (%): 98 / 88
- Tumor types
 - » GI (N=24)
 - » GYN (N=9)
 - » GU (N=3)
 - » Sarcoma (N=2)
 - » Other (N=3)
- Total of 209 doses administered to date
- Average # of doses/patient: 5.1 (range 1-28)
 - » Includes one patient continuing on study after >1 year of dosing

Treatment Emergent Adverse Events

Number of Patients with Grades 1, 2, 3, 4

Toxicity**	% of Patients (n=41)	ALN-VSP02 Dose Level (mg/kg)						
		0.10 (n=3)	0.20 (n=3)	0.40 (n=6)	0.70 (n=7†)	1.00 (n=9)	1.25 (n=11‡)	1.50 (n=2)
Fatigue	24%	1, 0, 0, 0	0, 0, 0, 0	0, 2, 0, 0	2, 1, 0, 0	1, 0, 0, 0	1, 1, 0, 0	1, 0, 0, 0
Nausea	17%	1, 0, 0, 0	0, 1, 0, 0	1, 1, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Fever	15%	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	2, 0, 0, 0	2, 0, 0, 0	0, 0, 0, 0
Infusion-Related Reaction	15%	0, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 1, 0, 0	0, 3, 0, 0	0, 1, 0, 0	0, 0, 0, 0
Vomiting	12%	2, 0, 0, 0	1, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
AST Elevation	12%	0, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 1*, 0	2, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Rash/Flushing	12%	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Thrombocytopenia	12%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 1*, 0	2, 0, 0, 0	0, 0, 2, 0	0, 0, 0, 0
Chills/Rigors	10%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 2, 0, 0	0, 0, 0, 0
Diarrhea	7%	0, 0, 0, 0	0, 1, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Abdominal Pain	7%	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Headache	7%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Hypertension	5%	0, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
ALT Elevation	5%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 1*, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Dizziness	5%	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Anorexia	5%	1, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

**Includes only those toxicities deemed related or possibly related to study drug in 5% or more pts

*Patient with extensive liver metastases died from liver failure, possibly related to study drug

†1 patient reduced to 0.40 mg/kg after dose 1

‡2 patients reduced to 1.00 mg/kg after dose 1

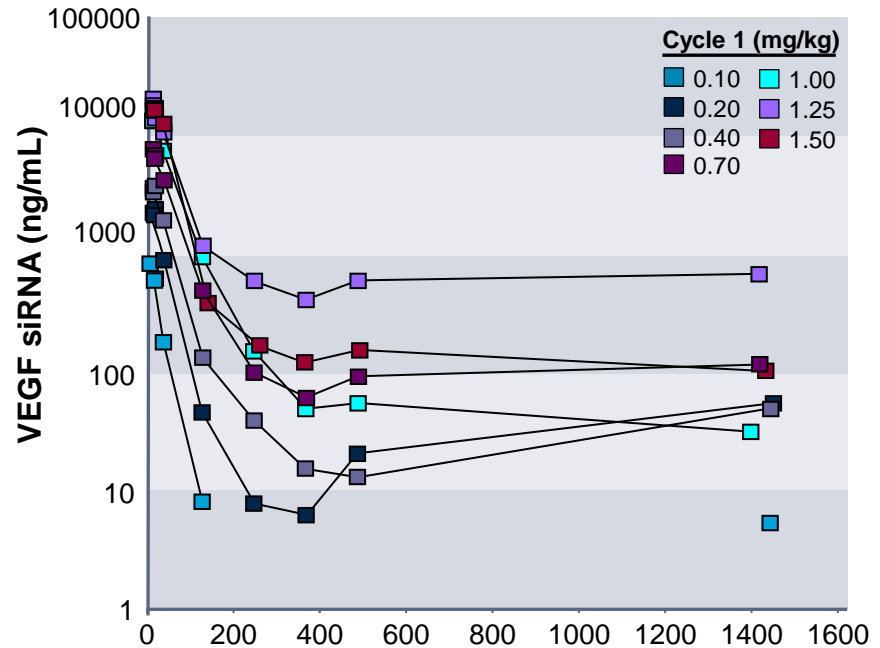
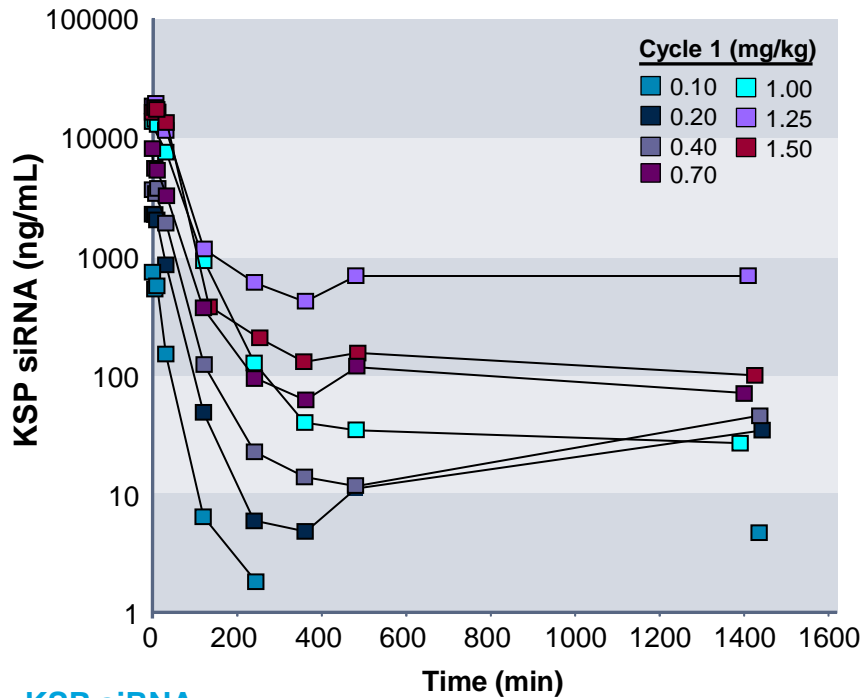
Serious Adverse Events

Patient #	Tumor Type	Dose Level (mg/kg)	Event(s)	Relatedness
003-012	Pancreatic neuroendocrine	0.70	<ol style="list-style-type: none"> 1. Liver failure 2. Hepatic encephalopathy <p>Both arising 5 days post-dose 2, resulting in death</p>	Possibly related
001-021	Endometrial	0.70	<ol style="list-style-type: none"> 1. Elevated WBC 2. Fever <p>Both occurred post-dose 23. Hospitalized to rule out infection and then resumed dosing on study.</p>	Possibly related
001-030	Endometrial	1.25	<p>Elevated WBC post-dose 3. Hospitalized to rule out infection and then resumed dosing on study.</p>	Possibly related

- 5 possibly related SAEs in 3 patients
- 23 unrelated SAEs in 10 patients

Plasma PK Data Summary

VEGF and KSP siRNAs



KSP siRNA

ALN-VSP Cycle 1 (mg/kg)	0.10 (n=3)	0.20 (n=3)	0.40 (n=5)	0.70 (n=7)	1.00 (n=4)	1.25 (n=5)	1.50 (n=2)
C_{max} ($\mu\text{g/mL}$)	0.76 ± 0.36	2.26 ± 0.54	3.92 ± 1.12	7.99 ± 3.39	15.31 ± 5.35	21.94 ± 7.64	18.04
AUC_{0-last} ($\text{min} \cdot \mu\text{g/mL}$)	30.83 ± 20.86	133.02 ± 46.66	252.38 ± 97.9	523.18 ± 206.18	923.59 ± 360.13	2035.74 ± 1100.08	1491.03

VEGF siRNA

ALN-VSP Cycle 1 (mg/kg)	0.10 (n=3)	0.20 (n=3)	0.40 (n=5)	0.70 (n=7)	1.00 (n=4)	1.25 (n=5)	1.50 (n=2)
C_{max} ($\mu\text{g/mL}$)	0.86 ± 0.42	2.51 ± 0.56	4.31 ± 1.10	8.81 ± 2.75	15.84 ± 7.01	21.58 ± 6.80	18.19 ± 1.09
AUC_{0-last} ($\text{min} \cdot \mu\text{g/mL}$)	68.95 ± 66.12	143.11 ± 58.58	272.21 ± 98.16	626.37 ± 272.66	984.26 ± 436.81	2006.58 ± 1113.54	1514.63 ± 150.24

- AUC and C_{max} dose proportional
- Same PK profile for Cycles 1 and 2 with no evidence of drug accumulation

Safety Summary

- ALN-VSP was generally well-tolerated
 - » 1 patient has so far received 28 doses over >1 year
- No dose-dependent changes in liver function tests
- Grade 1-2 fatigue (24%), nausea (17%) and fever (15%) most common AEs with no clear dose dependence
- Grade 2 infusion-related reactions seen in 15% of patients or 3% of doses administered
 - » Responded to slowing of infusion
 - » No patients discontinued from study because of infusion reaction
- Grade 1-2 chills/rigors seen at 1.25 mg/kg dose level
 - » Occurred after completion of dosing in 3 of 11 patients and associated with higher levels of transient IL-6 induction (peak at 6 hrs post-dose, resolution by 24 hrs)
 - In 2 patients, symptoms re-occurred after dose reduction to 1.0 mg/kg
- Dose-limiting toxicities included:
 - » Liver failure and death in patient with extensive hepatic metastases and prior splenectomy/partial hepatectomy at 0.7 mg/kg (possibly related),
 - » Transient grade 3 thrombocytopenia in 2 patients at 1.25 mg/kg,
 - » Grade 3 hypokalemia in 1 patients at 1.5 mg/kg
- Recommended Phase II dose is 1.0 mg/kg IV q2 weeks

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Tumor Response Summary

Dose Level (mg/kg)	N (evaluable for response)	Avg # of Doses Received (range)	# Pts with Stable Disease or Better for ≥ 2 mos	# Pts Who Went on to Extension Study (>8 doses) to Date
0.10	3	3 (2-4)	0	0
0.20	3	4 (4-4)	0	0
0.40	7*	4.6 (2-11)	1	1
0.70	5	9.6 (3-23)	3 (includes 1 PR with ~70% tumor reduction)	2
1.00	11†	6.7 (2-12)	7	4
1.25	7	2.4 (1-6)	2	0
1.50	1	4	0	0

PR: partial response

*Includes 1 patient whose first dose was given at 0.7 mg/kg

†Includes 2 patients whose first dose was given at 1.25 mg/kg

Stable disease or better in:

- 1/13 pts (8%) at ≤ 0.4 mg/kg
- 12/24 pts (50%) at ≥ 0.7 mg/kg

Characteristics of Patients with Disease Control

Patient #	Dose Level (mg/kg)	Tumor Type	Best Response	# of Doses
016	0.40	Squamous Cell Head and Neck	SD	11
019	0.70	Colorectal	SD	8
020	0.70	Angiosarcoma	SD	10
021	0.70	Endometrial	PR	28 (ongoing)*
022	1.00	Colorectal	SD	8
025	1.00	Synovial Sarcoma	SD	7
037	1.00	Renal Cell	SD	12 (ongoing)*
040	1.00	Pancreatic Neuroendocrine	SD	11 (ongoing)*
041	1.00	Renal Cell	SD	9 (ongoing)*
042	1.00	Uveal Melanoma	SD	6
044	1.00	Pancreatic Neuroendocrine	SD	8 (ongoing)*
028	1.25	Esophageal Adenocarcinoma	SD	6
030	1.25	Endometrial	SD	4

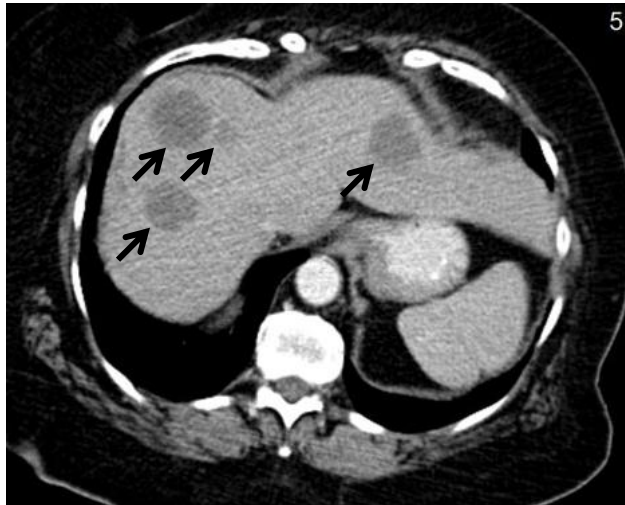
SD: stable disease
PR: partial response

*Patients remaining on extension study

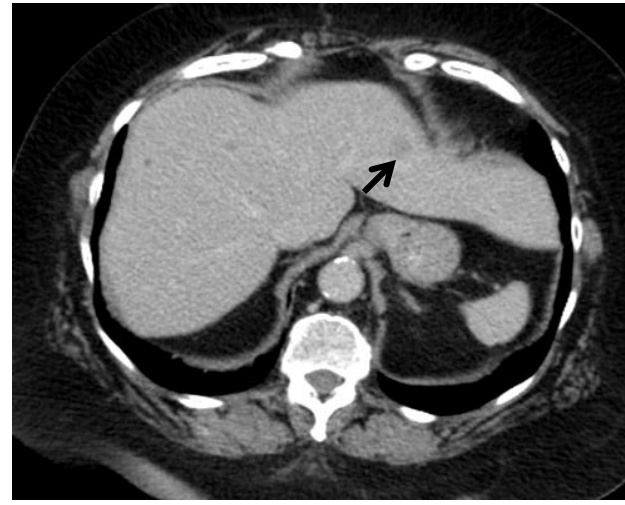
Major Response in Endometrial Cancer

Patient 021, 70% Regression of Liver Metastases

Pre-Treatment



After 12 Doses ALN-VSP*

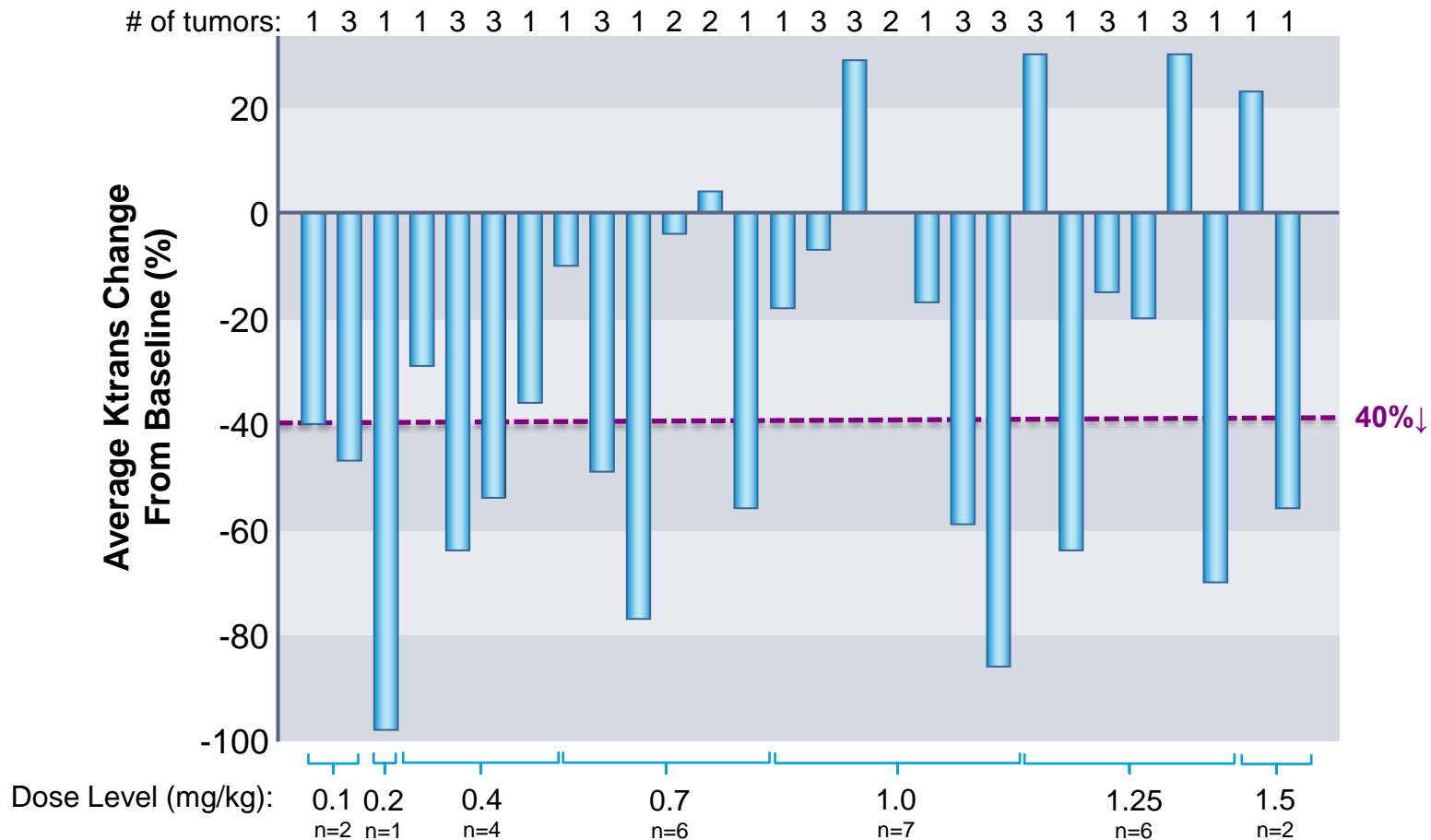


*Response ongoing after 24 doses

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DCE-MRI Results on Evaluable Patients



- 46% (13/28) of patients with average Ktrans ↓ of ≥40%

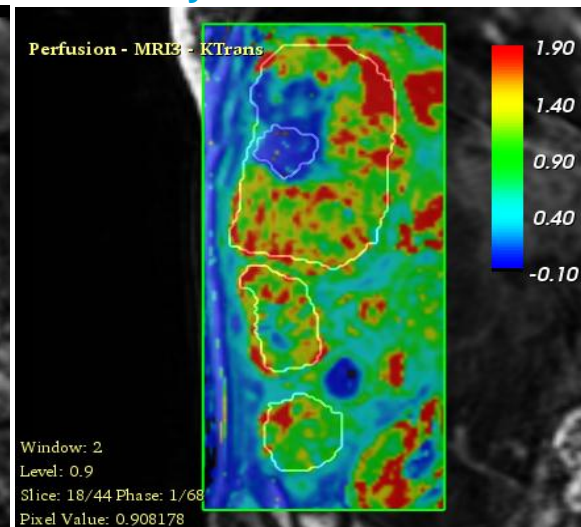
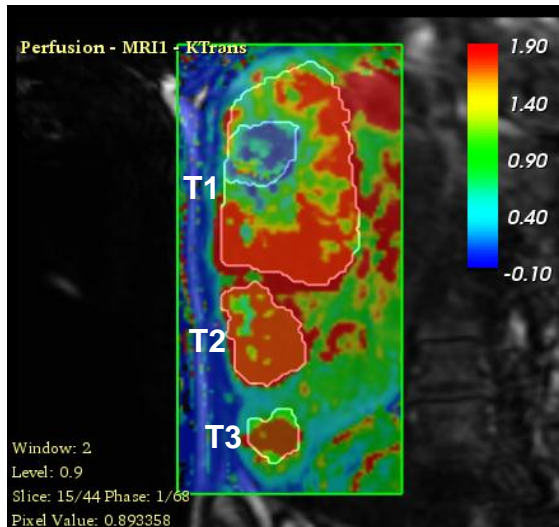
ASCO, June 2011

DCE-MRI in PNET Patients with Liver Metastases

Pre-Treatment

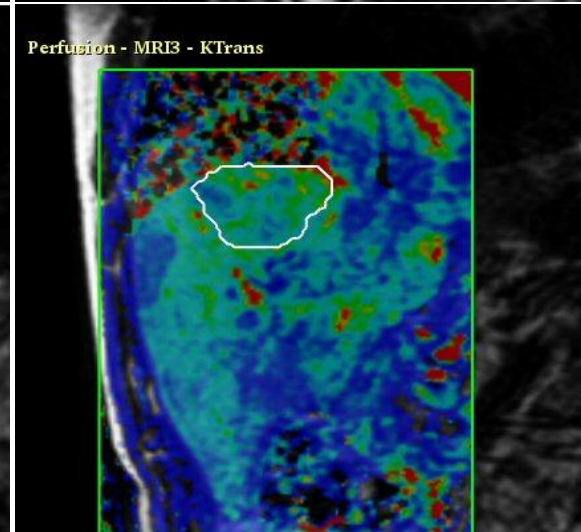
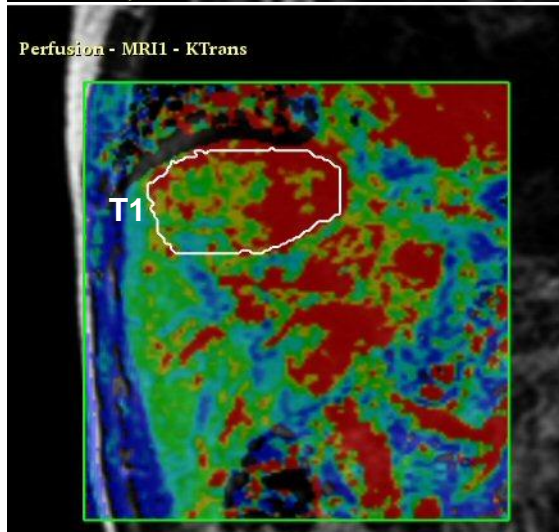
Day 7 Post-Dose 1

Pt 012
(0.7 mg/kg)



Avg Ktrans↓=49%
(n=3 tumors, T1-T3)

Pt 044
(1.0 mg/kg)



Avg Ktrans↓=86%
(n=3 tumors, only T1 shown)

T: liver tumor
PNET: pancreatic neuroendocrine tumor

Voluntary Tumor Biopsies

- CT-guided core needle biopsies obtained pre- and post-dose 1 in patients on voluntary basis
 - » Analyses:
 - Drug levels
 - 5' RACE
 - qPCR
- 29 Tumor biopsies obtained from 15 patients across multiple dose levels
 - » 0.4 mg/kg (n=3), 0.7 mg/kg (n=2), 1.0 mg/kg (n=6), 1.25 mg/kg (n=3), 1.5 mg/kg (n=1)
 - » Liver tumor biopsies in 11 patients
 - » Extrahepatic tumor biopsies in 4 patients
- Histological exam reveals high degree of variability in proportion of tumor, fibrotic/necrotic tissue, and normal tissue in biopsy samples
 - » Impacts certain quantitative interpretations of molecular results

Drug Levels in Tumor Biopsies Post-Dose 1

Pt #	Dose mg/kg	Tumor Type (Biopsy Site, Day of Post-Dose Biopsy)	Post-Dose Biopsy (%)			Drug Levels** (ng/g tissue)	
			Viable Tumor	Liver	Fibrosis/ Necrosis	VEGF siRNA	KSP siRNA
007	0.40	Colorectal (liver, d7)	17	80	3	25.7	12.5
017	0.40	Ovarian (liver, d2)	0	95	5	28.9	17.2
019	0.70	Colorectal (liver, d2)	20	5	75	142	73.3
022	1.00	Colorectal (adrenal, d2)	10	0	78*	9.8	3.8
025	1.00	Sarcoma (muscle, d2)	96	0	4	0.45	0.40
033	1.00	Colorectal (liver, d3)	56	0	35†	5.4	2.2
041	1.00	RCC (liver, d2)	70	15	15	6.0	6.8
042	1.00	Uveal mel (liver, d2)	100	0	0	7.0	5.8
026	1.25	Colorectal (liver, d6)	14	0	71‡	0.32	<LLOQ
031	1.25	Ovarian (abdomen, d4)	30	0	70	4.9	3.6
035	1.25	Ovarian (L. node, d5)	40	0	60	20.4	20.3
032	1.50	Small bowel (liver, d6)	42	0	58	4.9	3.6

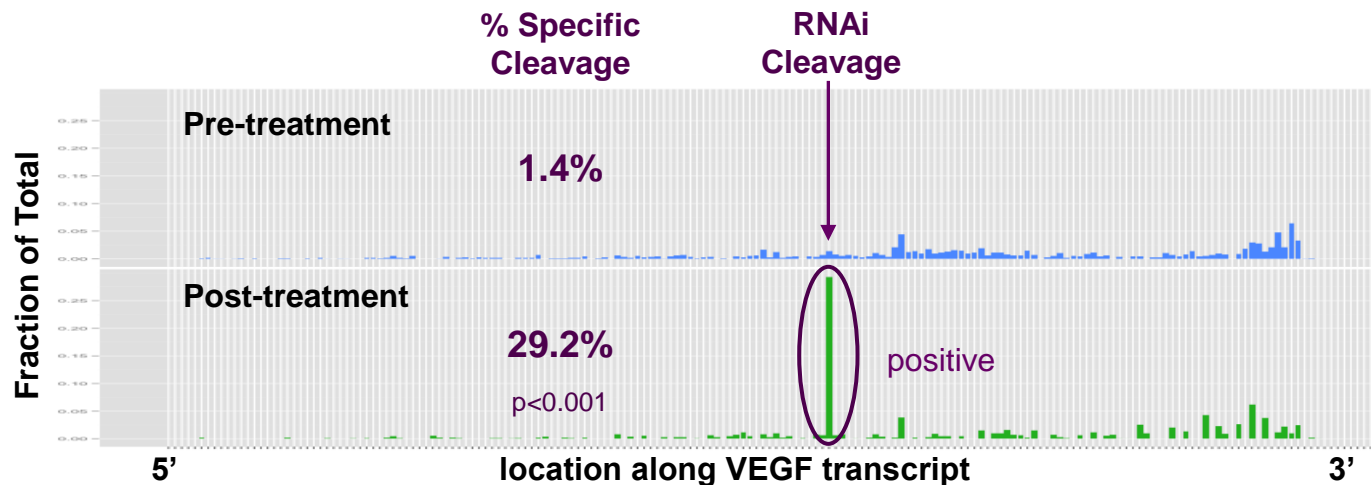
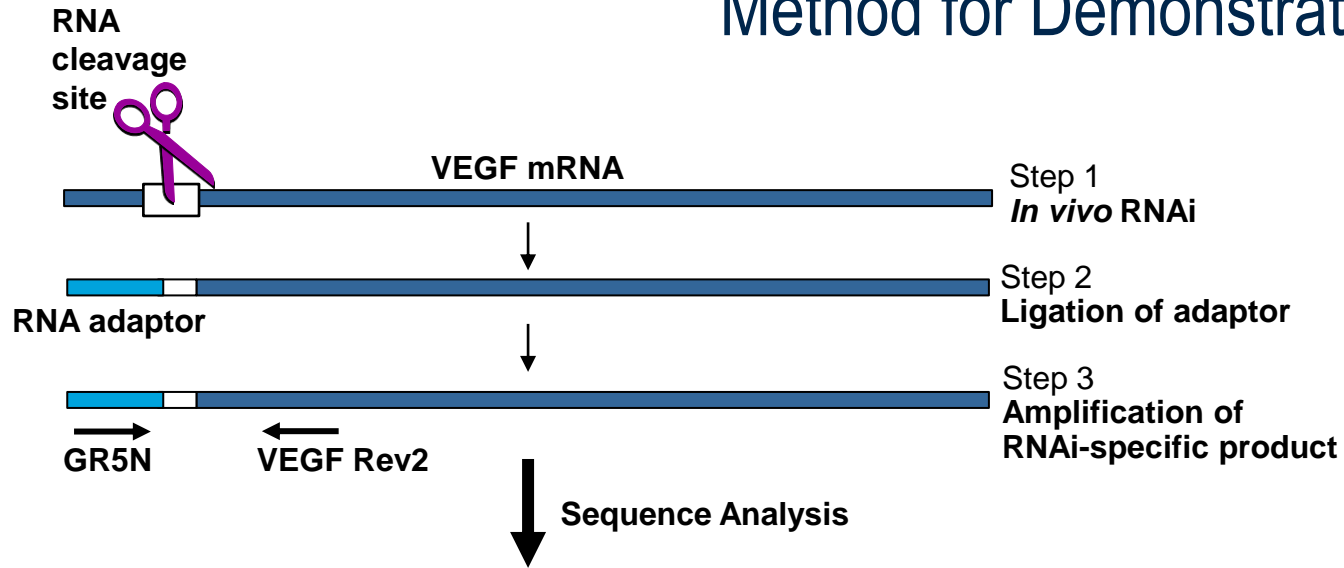
**Measured by qPCR, LLOQ (lower limit of quantitation)=0.14 ng/g tissue. All pre-treatment biopsy samples were <LLOQ.

*Remaining 12% was normal adrenal (6%) and fat (6%)

†Remaining 9% was skeletal muscle

‡Remaining 15% was skeletal muscle

VEGF 5' RACE Assay Method for Demonstrating RNAi



Illustrative
Example:
Patient 016

Tumor Biopsies Positive By VEGF 5' RACE

Pt #	Dose mg/kg	Tumor Type (Biopsy Site, Day of Post-Dose Biopsy)	Pre-Dose Biopsy (%)			Post-Dose Biopsy (%)			VEGF 5' RACE
			Viable Tumor	Liver	Fibrosis/Necrosis	Viable Tumor	Liver	Fibrosis/Necrosis	
016	0.40	SCC H&N (liver, d2)	10	0	90	0	100	0	Pos*
017	0.40	Ovarian (liver, d2)	N/A	N/A	N/A	0	95	5	Pos*
031	1.25	Ovarian (abdomen, d4)	25	0	75	30	0	70	Pos*

SCC H&N: Squamous cell cancer of head and neck
 N/A: No sample for analysis

*p<0.001

- 15 patients with post-treatment biopsy evaluable by 5' RACE :
 - » 0.4 mg/kg (n=3), 0.7 (n=2), 1.0 (n=6), 1.25 (n=3), 1.5 (n=1)
- 3 of 15 positive for VEGF 5' RACE
 - » 2 liver tumor biopsies at 0.4 mg/kg
 - » 1 extrahepatic tumor biopsy at 1.25 mg/kg
- Assay development in progress for KSP 5' RACE
 - » Required due to low KSP mRNA levels

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Clinical Activity/PD Summary

Evidence of antitumor activity in heavily pre-treated patients at doses ≥ 0.4 mg/kg

- Overall, 13 of 31 evaluable patients (42%) with disease control (SD or better) at doses of 0.4-1.5 mg/kg
 - » Average duration of disease control to date is approximately 5 months (range 2-14)
- Major ongoing response (PR with $\sim 70\%$ tumor reduction) in patient with endometrial cancer and multiple liver metastases treated at 0.7 mg/kg
 - » Patient treatment continuing on extension study after >1 year
- 64% (7 of 11) of patients with stable disease ≥ 2 months at recommended Phase II dose of 1.0 mg/kg
 - » Four patients with stable disease for 4-6 months continue to receive drug on extension study

DCE-MRI results suggestive of anti-VEGF effect

- $\sim 50\%$ of pts showing $\geq 40\%$ drop in Ktrans in liver tumors, including endometrial cancer patient with major response (PR) and two PNET pts

siRNA delivery and RNAi proof of mechanism shown in tumor biopsies

- Pharmacologically relevant concentrations of VEGF and KSP siRNAs in hepatic and extrahepatic tumors
- Molecular evidence of RNAi-mediated VEGF mRNA cleavage in biopsies of hepatic and extrahepatic tumors by 5' RACE